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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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The effect of air pollutants on airway innate immune cells in patients with asthma

To the Editor,

Air pollution is becoming a serious health risk to patients with chronic diseases, especially asthma. Particulate matter (PM), diesel exhaust particles (DEP), ozone (O_3), and other chemicals and pollutants have been shown to have a detrimental effect on respiratory function.[1] However, the mechanisms through which each air pollutant aggravates asthma symptoms are not clearly understood.

Innate lymphoid cells (ILCs) have gained attention due to their potent role in regulating allergic disorders by producing diverse cytokines in response to different stimuli.[2] In a previous study, we demonstrated that ILCs can regulate asthma phenotypes by interacting with other innate immune cells in human asthmatics.[3] In the current study, we aimed to determine (a) how ILCs and macrophages respond to various air pollutants and (b) how these cells affect asthma symptoms.

A total of 118 subjects (50 healthy subjects, 52 mild asthma patients, and 16 severe asthma patients) were recruited (Table S1), and innate immune cells including ILCs and macrophages were analyzed in the induced sputum (Figure S1). Initially, ILCs defined as $CD45^+$, lineage-negative (Lin^-) cells that express CD127. To subdivide ILC populations, ST2, c-Kit, and NKp44 makers were used. $CD45^+CD68^+$ cells were considered as macrophages, and M1, M2, and alveolar macrophages were further identified by CD11c and CD206 expression (Figure S1A and B). First, to verify the impact of air pollutants on asthma, we analyzed the relationship between the concentrations of air pollutants [particulate matter with a diameter ≤ 10 μm (PM_{10}), particulate matter with a diameter ≤ 2.5 μm ($PM_{2.5}$), Ozone (O_3), nitrogen dioxide (NO_2), carbon monoxide (CO), and sulfur dioxide (SO_2)] measured in the residential area of the patients on the day of getting sputum, asthma symptom scores, and lung function measurements from participants, including healthy controls (Table S2). Contrary to general expectations, most air pollutants had little or no effect on the asthma index. Among the various air pollutants, only the concentration of PM_{10} in their residential area correlated with the asthma control questionnaire (ACQ) and the asthma control test (ACT) scores. The ratio of forced expiratory volume in 1 second (FEV_1) to forced vital capacity (FVC), a critical indicator of asthma, was not affected by air pollutants except for NO_2 (Table S2). Based on the hypothesis that air pollutants might affect asthmatics more strongly than healthy controls, we reanalyzed the effect of air pollutants specifically in patients with asthma (Figure 1A-F). In patients with asthma as well, PM_{10} in their residential area had the greatest impact on ACQ and ACT.

Next, we examined the correlation between the concentration of air pollutants and the frequency of ILCs and macrophages (Table S3). Linear regression analysis showed that only the frequency of ILC2s

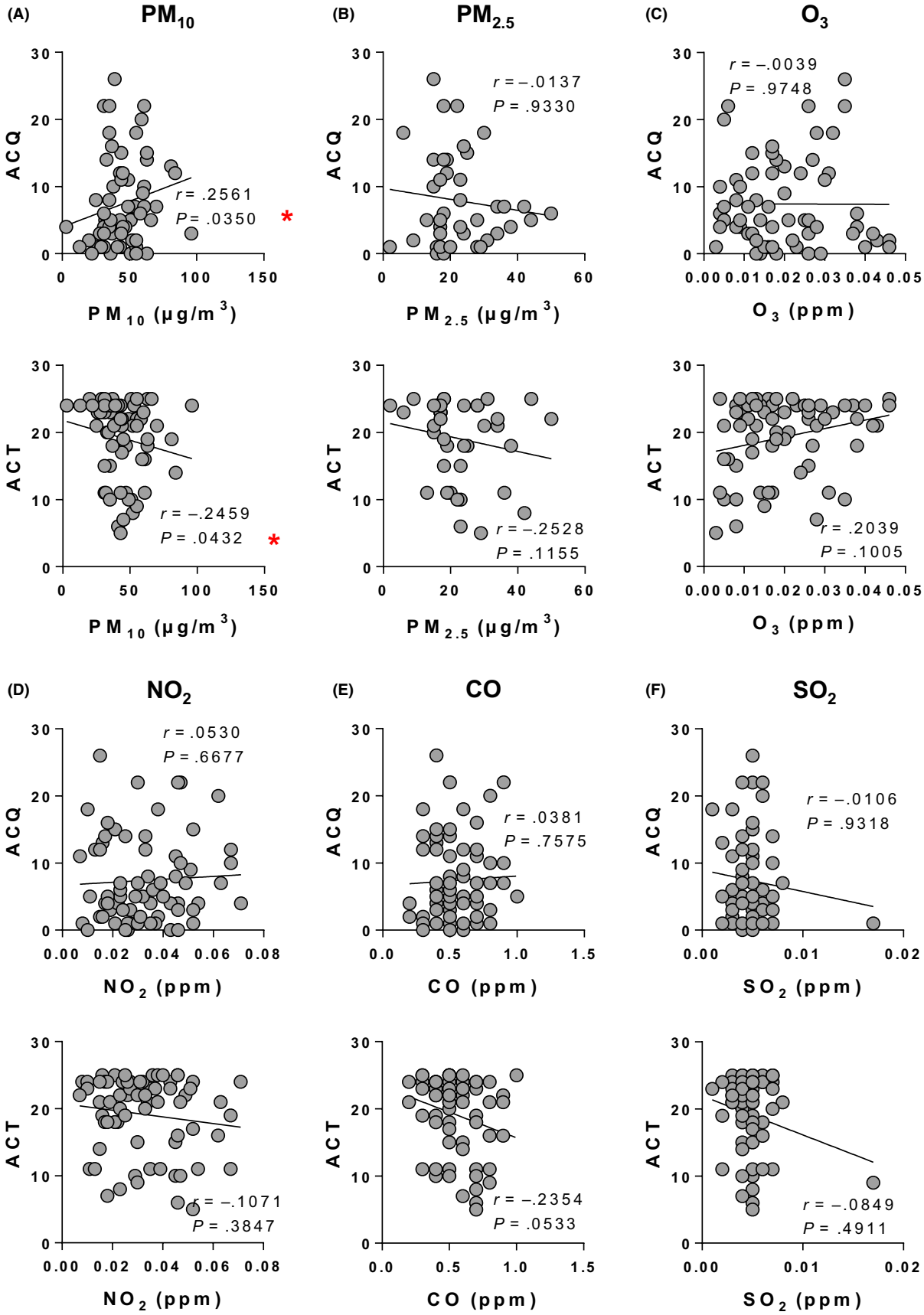
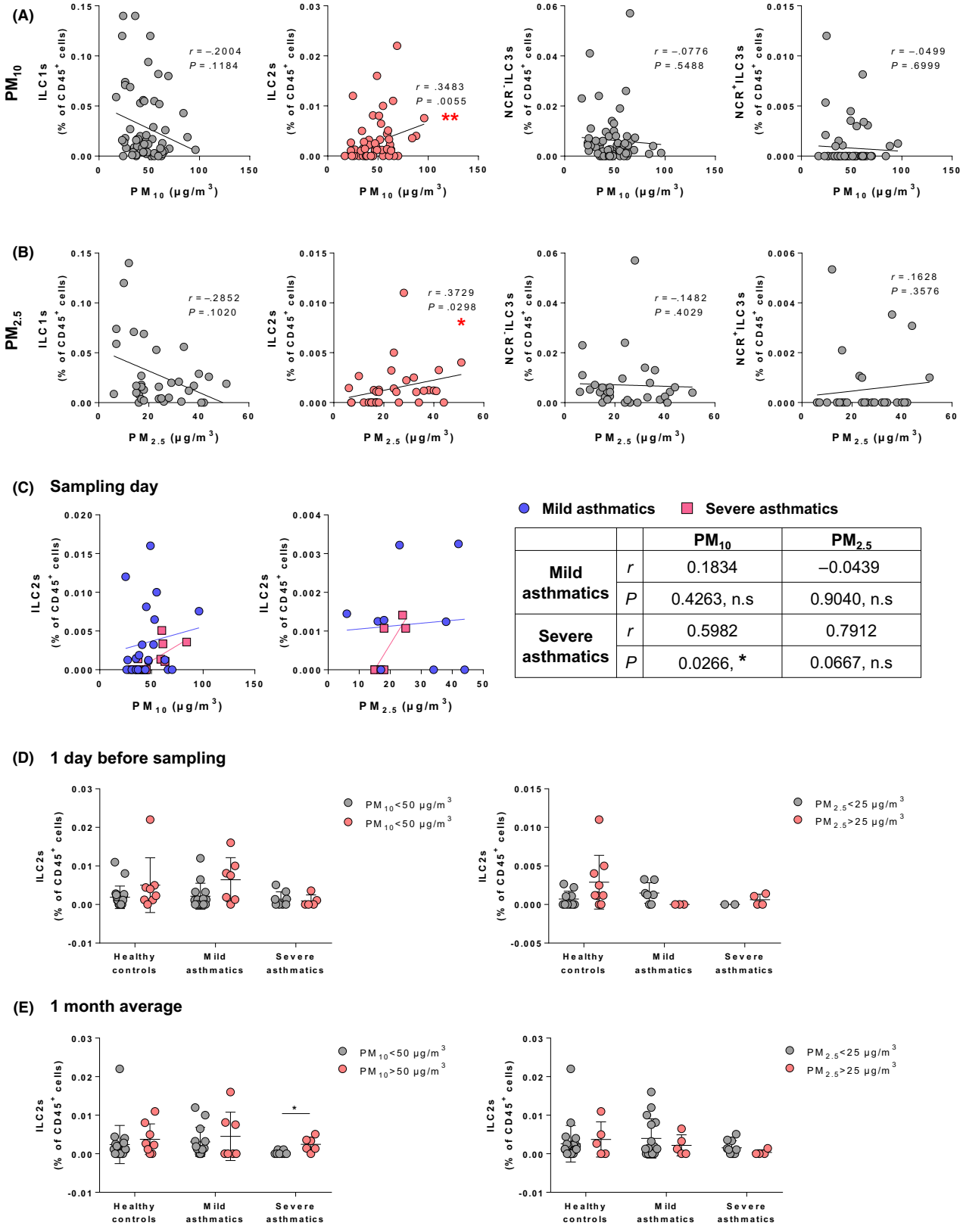


FIGURE 1 PM_{10} level has correlation with indexes of asthma control of asthmatics. A-F, Correlation of PM_{10} (A), $PM_{2.5}$ (B), O_3 (C), NO_2 (D), CO (E), and SO_2 (F) level, measured at the sampling day with ACQ and ACT scores.*$P < .05$, correlation coefficient calculated using Spearman's correlation test



		PM_{10}	$PM_{2.5}$
Mild asthmatics	*r*	0.1834	−0.0439
	P	0.4263, n.s	0.9040, n.s
Severe asthmatics	*r*	0.5982	0.7912
	P	0.0266, *	0.0667, n.s

FIGURE 2 PM level has correlation with ILC2s in induced sputum of severe asthmatics. A-B, Correlation coefficient of each group of ILCs in induced sputum of total subjects with PM_{10} (A) or $PM_{2.5}$ (B) level, measured at the day of sampling in their residential area. C, Correlation of PM_{10} (left panel) or $PM_{2.5}$ level (right panel) with ILC2s from induced sputum of mild asthmatics or severe asthmatics. Table showed the correlation coefficient values. D, Comparison of ILC2s according to PM_{10} (left panel) or $PM_{2.5}$ level (right panel) measured at a day before sampling. E, Comparison of ILC2s according to average PM_{10} (left panel) or $PM_{2.5}$ level (right panel) of a month of sampling. *$P < .05$; **$P < .01$; n.s, nonsignificant; calculated using correlation coefficient calculated using Spearman's correlation test. values represent the mean ± SD

correlated positively both with PM_{10} and $PM_{2.5}$ levels of their residential area. The frequency of ILC1s correlated with the level of O_3, NO_2, or CO, but did not correlate with the level of PM. The frequency of alveolar macrophages (AMs) negatively correlated with O_3 and positively correlated with SO_2, but did not correlate with the other air pollutants, including PM. Furthermore, there was no correlation between the frequency of M1 or M2 macrophages and the level of air pollutants.

As ILCs in the airway were more affected by PM than macrophages, we focused on the differences in ILCs according to the PM concentration. A correlation analysis between the PM_{10} or $PM_{2.5}$ level of their residential area and the percentage of ILC2s in the sputum showed a positive relationship in all subjects (Figure 2A and B). The proportion of other ILCs, ILC1s or ILC3s, did not show significant correlation with PM level. When asthmatics were subdivided according to disease severity, only severe asthmatics showed a significant positive correlation between the proportion of ILC2s and the PM_{10} level (Figure 2C). To determine whether the effect of PM exposure on ILCs is acute or chronic, we compared the proportion of ILC2s with the PM level measured at the residential area of the subjects 1 day before sampling and with the average PM level in the month before sampling (Figure 2D and E). ILC2s were significantly increased when severe asthma patients were exposed to high PM_{10} level for 1 month. $PM_{2.5}$ exposures also did not affect the proportion of ILC2s both acutely and chronically. This result suggests that ILC2s respond rapidly to PM_{10}, which might induce more severe symptoms in asthmatics. Other subsets of ILCs were not changed according to PM exposure (Figure S2A-B). The frequency of ILC2s was not changes by medications, so we could rule out the effect of medications on ILCs (Table S1 and Figure S3A-H). Moreover, macrophages, including M1 and M2 macrophages, were also not affected by the PM_{10} or $PM_{2.5}$ concentrations (Figure S4A-B).

How various air pollutants modulate different airway innate immune cells is an important question that could lead to insight into the mechanism through which air pollution exacerbates asthma. To the best of our knowledge, this is the first human study to confirm an association between air pollutants and airway innate immune cells in asthma. The PM_{10} concentration of the residential area of the patients positively correlated with not only the asthma symptom score, but also with the proportion of ILC2s in the sputum. However, current data have limitations that do not reflect actual exposure of air pollutants during the observation period (eg, indoor pollutants, exposure to the residential environment, and exposure at the working place). Although direct evidence is limited, our results suggest that exposure to PM_{10} exacerbates asthma symptoms in severe asthmatics and that ILC2s may be involved in this process. Studies in mice have revealed that DEP, O_3, and multi-wall carbon nanotubes activate ILC2s to produce IL-5 and IL-13 in response to IL-33 produced by airway epithelial cells.[4] In the current study, only PM_{10} levels positively correlated with the proportion of ILC2s. However, previous studies showed that O_3, a potent oxidizing agent, induced type 2 cytokine secretion from ILC2s.[5] We did not see any effect of O_3 on the percentage of ILC2s, but the O_3 concentration did positively correlate with the frequency of ILC1s.

Macrophages are first-line responders to exogenous particles. In previous studies, in vitro stimulation of AM with PM or fine particles increased the production of inflammatory cytokines such as IL-6 and IL-8, and the monocyte chemoattractant MCP-1.[6,7] However, in vivo inhalation of PM does not alter the infiltration or polarization of macrophages in the lung.[8,9] Our data also showed that there was no correlation between the level of air pollutants and the frequency of macrophages in the airway. Since the current study is a cross-sectional study using indirect variables, including public air quality data, there are limitations to interpreting these results to indicate a direct effect of PM on immune cell composition and phenotype. Nevertheless, our results suggest that certain air pollutants can affect innate immune cells in the airway, exacerbating asthma symptoms. Given that different air pollutants affect innate immune cells differently, it would valuable to expand our findings to other diseases associated with air pollution.

In conclusion, these results add epidemiologic evidence for the impact of air pollution on airway innate immune cells, especially ILC2s, in human asthma. These results may explain the mechanism through which air pollution exacerbates asthma. To prevent exacerbation of asthma by air pollutants, further studies on how air pollutants change the function of immune cells in the airway, particularly ILCs, are needed.

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CONFLICTS OF INTEREST

The authors declare no potential conflicts of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Two-year drug survival of dupilumab in a large cohort of difficult-to-treat adult atopic dermatitis patients compared to cyclosporine A and methotrexate: Results from the BioDay registry

To the Editor,

Dupilumab is a fully human monoclonal antibody that targets the interleukin (IL)-4 receptor subunit α (IL-4 Rα), the common subunit of the type 2 cytokines IL-4 and IL-13, blocking signaling of both cytokines and consequently inhibiting the entire Th2 pathway.[1] Overall, the clinical efficacy and safety of dupilumab ± topical corticosteroids